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NET-NECROSIS OF IRISH POTATO TUBERS

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1. Net-necrosis is a disease of potato tubers and is recognized by the occurrence of brown thread-like lines or streaks which originate at the stem end and form a network beneath the skin often extending well toward the apex of the tuber.

2. Net-necrosis is widely distributed in potato-growing regions and is distinguished from other tuber discolorations in that the browning is confined to the phloem tissues and that the disease develops during the storage period.

3. Leafroll and net-necrosis are shown to have a direct relationship, since necrosis is a first-season symptom following initial leafroll infection.

4. Changes in necrotic tuber tissues involve the accumulation in them of lignin, suberin and pectic substances.

5. Necrotic spots or areas become separated from the healthy tuber tissue by the formation of new cork-cambium walls.

6. Net-necrosis was experimentally produced by the transferring of leafroll virus by means of aphids from leafroll to healthy plants whose tubers subsequently developed necrosis. These necrotic tubers when germinated produced leafroll plants.

7. Since net-necrosis is a result of leafroll infection, control measures should be directed toward the elimination of leafroll. The procedure should be the elimination of diseased plants by the use of a tuber unit seed plot and the growing of seed stock in small fields isolated from sources of infection.

BULLETIN 289: NET-NECROSIS OF IRISH POTATO TUBERS[1]

ALFRED H. GILBERT

REVIEW OF LITERATURE

NET-NECROSIS

The increasing prevalence in certain potato-growing sections of the eastern United States of a tuber necrosis which develops during the storage period and which, in certain strains of seed and under certain environmental conditions, constitutes a serious menace to potato culture, furnishes the challenge for the present study.

This tuber necrosis, commonly known as net-necrosis, is here shown to be a true phloem necrosis since the writer's histological studies have demonstrated that it is the phloem tissues of the tuber that are affected. In view of this revelation it is evident that the designation "phloem-necrosis," originated by Quanjer (42) in 1913 and applied to conditions of the stems and foliage of leafroll plants, is also applicable to this disease of the potato tuber. The term net-necrosis, not particularly appropriate when used to describe the appearance of the discolored tissues as observed in tuber cross-section (plate III, fig. 1), is more accurately descriptive of the appearance of the network of browned primary phloem strands in the region of the outer phloem when this tissue is exposed by the removal of the skin and outer cortical layers (text fig. 1).

W. A. Orton (36) first published a description of net-necrosis in 1914, stating that this tuber disease had been under observation by him for some five or six years and that the name was suggested by Wollenwebber. The disease is here described as an internal browning, characterized by the "occurrence of narrow streaks or dots of browned tissue outside of the vascular ring and extending from the stem end into the tuber for a considerable distance or entirely through it." The cause was said to be unknown.

[1]The writer gratefully acknowledges indebtedness to the station plant pathologist, Dr. B. F. Lutman, and to Professors L. R. Jones, C. E. Allen and E. J. Kraus of the University of Wisconsin for advice and encouragement, also to the authorities of the University of Wisconsin for laboratory facilities and other courtesies.

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In 1911, McAlpine (31) lists among the diseases of the potato the following common causes of missing hills: "thready-eye," "needle-eye" and "spindle-disease." It is further suggested that the grower may recognize thready-eye in several ways, as follows: (a) by the occurrence of slender, pale shoots; (b) by the presence of minute tubers close to the eye without shoots; and (c) by a "network of dark streaks throughout the entire flesh of the tuber." These combined symptoms, particularly those of (a) and (c), make it reasonably clear that the trouble referred to as a matter of fact was net-necrosis and that this malady was associated, as is usually the case, with the development of spindling sprouts.

C. R. Orton (37), in 1916, notes the occurrence of "internal spotting and streaking of potato tubers," particularly in dry seasons, and adds that "net-necrosis was unusually prevalent in Pennsylvania in 1914." It was thought that the two troubles mentioned were associated with certain soil conditions, such as lack of moisture and of potash. Cook (9) refers to net-necrosis as a "disease of unknown origin appearing as dark, brownish zones or streaks which originate at the point of attachment and extend toward the apex." These discolorations were said to be sometimes mistaken for Fusarium or Verticillium wilt. Some evidence indicated that the disease was transmitted in the seed and, further, that it was often absent at digging time but developed during storage.

In 1921, Schultz and Folsom (49, p. 78) expressed the view that "net-necrosis is apparently a leafroll symptom, being a discoloration which results from tuber phloem-necrosis and which appears more often as conditions of variety, recency of infection and weight of tuber are more favorable." Their studies further show that net-necrosis is never present in lots free from leafroll and is restricted to the tubers transmitting leafroll. These results are substantiated in a later paper by the same authors (50).

Kasai (26), in 1921, reporting on leafroll studies, states that discolorations of the vascular bundles were seen in "infected tubers which were stored for a while" and suggests that probably this trouble is "identical with the net-necrosis described by Schultz and Folsom."

Quanjer (45, pp. 6, 7) calls attention to the fact that it is often difficult, depending upon the usual symptoms, to recognize leafroll, and that in seeking for a more accurate and constant characteristic he found phloem-necrosis. He further adds: "Schultz and Folsom claim to have found net-necrosis and spindling-sprout as characteristics of

true leafroll, but I have not observed these as regular symptoms, though it may be that net-necrosis develops in leafroll infested tubers of some varieties, which, instead of being planted in the spring, are kept very long in cool storage."

Observations showing correlations between leafroll, net-necrosis and spindling-sprout were recorded in an earlier paper (17) by the present writer. These results were in harmony with the previous reports of Schultz and Folsom and with those of Kasai and left little doubt as to a positive relation existing between net-necrosis of the tuber and leafroll of the foliage. None of the earlier studies, however, have dealt with the histology and cytology of the necrotic tuber tissues nor with the progressive development of the malady within the tuber. Moreover the matter of the initiation of the necrotic symptoms has been largely one of conjecture, except that Schultz and Folsom (49, p. 69) note the influence of proximity to leafroll hills in connection with the production of necrosis in the field and also the significance of recency of infection in the same connection. In the light of certain experimental evidence secured in the present study, the observations noted above are of particular interest and importance.

While, as indicated by a review of the literature, considerable interest has been centered about the question of the diagnostic value for leafroll of pathological changes in the stems, such as lignification or obliteration of the phloem, comparatively little attention has been given to correlated tuber symptoms. Artschwager (4, p. 243), discussing "True Phloem-necrosis and the Leafroll Problem," says: "In the underground organs of diseased plants the phloem strands are usually normal, but in severe cases both stolons and tubers will show evidence of necrosis." It is assumed that Artschwager was speaking here of slight necrotic evidences in the phloem of stolons or tubers from leafroll plants and not of the characteristic net-necrosis of the tubers which is the subject of the present investigation.

Atanasoff (7) denied the existence of any correlation between leafroll and net-necrosis as reported in the United States by Schultz and Folsom (49) and the present writer (17), but claimed a demonstrated connection between a tuber necrosis and "aucuba mosaic." The illustration used by Atanasoff, as well as the description of his tuber necrosis, leave room for much doubt as to his claim that he was dealing with the trouble known in this country as net-necrosis. The statement (7, p. 935) that many hundreds of tubers from leafroll plants were examined with no case of net-necrosis to be seen, does not throw any light

upon the situation, for that is the result to be expected in our present understanding of the relations existing between leafroll and net-necrosis. However, a further statement is more significant, namely, that necrotic tubers were planted with no leafroll symptoms resulting in the progeny plants. Since this point has been repeatedly investigated by the writer and others with uniform results, that is to say, the production of leafroll plants from necrotic tubers in practically 100 percent of the cases, the writer is strongly of the opinion that the necrosis observed in the Holland material was not identical with that described by W. A. Orton and others in this country.

Schultz and Folsom, in discussing the relationship between net-necrosis and leafroll, have suggested (49, p. 64) that "net-necrosis, a tuber phloem-necrosis, is caused by the same virus as is leafroll or phloem-necrosis." Experimental evidence secured by the present writer furnishes strong proof of the correctness of this hypothesis.

OTHER TUBER DISCOLORATIONS

The student of potato diseases recognizes several tuber troubles, which, since they are characterized by internal tissue discolorations and necrotic areas, should be noted at this point and distinguished from the necrosis which is the subject of the present study. These are: internal brown spot (sprain, Eisenfleckigkeit); stem-end browning; frost-necrosis; and ring-disease (Kringerigheid).

Internal brown spot as recorded by Jones (24), Morse (32), Horne (23), W. A. Orton (36), Coons (10) and others, common in some parts of Europe but little known in America, seems to be quite distinct from net-necrosis. In the former the brown, necrotic tissue occurs in rounded or irregular areas and is variously distributed throughout the tuber, whereas in net-necrosis there is exhibited primarily a network of discolored strands which is confined to the regions of the phloem. Internal brown spot is believed by many to be associated with certain soil and fertilizer conditions. Furthermore it is not transmitted by the use of affected seed and may be controlled by proper cultural methods. Schwartz (51) and Horne (23) state that the diseased areas are surrounded by cork-cambium, a feature which is noted later as being characteristic of net-necrosis. Sorauer (53) describes a similar condition under the term "Eisenfleckigkeit"; Doidge (12) writes of "internal brown fleck" and Horne (23) discusses like symptoms under the names "internal disease" and "sprain." Paine (39), distinguishing between sprain and net-necrosis, attempts to show

that both are caused by the presence of a bacterial organism, *Pseudomonas solaniolens*. His results, however, are inconclusive.

Stem-end browning, which may be caused by any one of several fungi, such as Fusarium, Verticillium, etc., as described by Edson (14) or which, according to the same author, may occur in the absence of any organism, is a condition rather easily to be confused with the early stages of net-necrosis. In general the stem-end browning caused by fungi exists as a shallow region of discoloration in which the browned vascular tissues may resemble net-necrosis. In the case of net-necrosis, however, the stem-end discolorations, at first shallow, are progressive and gradually involve phloem tissues at greater distances from the basal end. Schultz and Folsom (49) refer to the difficulty of distinguishing between "wilt types of vascular discoloration" and net-necrosis and suggest that microscopic examination may be necessary. It will then be discovered that in the case of net-necrosis the phloem is the tissue affected, whereas in the browning due to wilt fungi it is the xylem which is involved.

Frost-necrosis as pictured and described by Jones, Miller and Bailey (25), and illustrated in plate III, fig. 2, is another tuber trouble the symptoms of which more or less resemble those of net-necrosis. Frost-necrosis is exhibited in three somewhat distinct types of tuber symptoms, viz., the "net," the "ring" and the "blotch" types. These several general types are often more diffuse than is the case in net-necrosis. The initial effects frequently shown in its earlier stages appear in intercellular spaces where browning occurs in the walls as a result of ice-crystal formation. Detailed histological studies of this and comparisons with other necrotic conditions have not yet been made by the writer.

A disease described under the name "Kringerigheid" has been observed and studied by Quanjer (46). It is a necrosis of certain tissues generally at or near the surface of the tuber, the discolorations being crescent or ring shaped, whence the name "ring disease" (Kringerigheid). Its cause is not known but it is considered by Quanjer as being due to some unknown organism, since contaminated soil could be freed from the causal agent by sterilization. The disease is affected by conditions of soil fertility and varietal differences of the potato. Quanjer uses the name "sprain" as synonymous with Kringerigheid, although the symptoms seem distinct from those of the disease termed sprain in England and Scotland as well as from those of internal brown spot in America.

LEAFROLL AND PHLOEM-NECROSIS

Since, as the writer has already noted in an earlier paragraph, and as Schultz and Folsom (49, p. 48) have stated, net-necrosis of the tuber is a necrosis of the phloem and associated with leafroll, it is pertinent to refer briefly to the symptomology of leafroll and to the origin of the term "phloem-necrosis," which now seems clearly to have a wider application than that proposed by Quanjer in 1913.

Quanjer states (45, p. 11) that he detected phloem-necrosis in 1908 and described it for the first time in 1913. It seems, however, according to W. A. Orton (36) that an outbreak of leafroll occurred in Europe in 1905 and recurred with virulence in 1907. From this time onward leafroll has been the subject of much investigation and numerous publications. In the United States the disease was first studied by W. A. Orton (36) in connection with outbreaks occurring in Colorado and western Nebraska in 1911 and 1912. During the earlier years its nature was entirely in doubt and suggestions as to its cause varied widely. The idea that it is a virus disease was suggested by Quanjer (43) in 1916. Extensive investigations by Schultz and Folsom (49), published in 1921, showed that it might be transmitted by grafting and also by aphids, that there was a definite relationship between leafroll and net-necrosis and also a correlation between necrosis and spindling-sprout. Quanjer (45) claims that the transmission of leafroll by aphids was shown by Oortwijn-Botjes (38) in 1920.

Significant results, particularly in the matter of insect transmission and starch accumulation in the leaves, have been reported by Murphy (33, 35), who found such accumulation to be constantly associated with leafroll. He concluded that the rolling of the leaves was a direct consequence of such an accumulation, but also found that certain other causes, such as stalk injury, produced a similar result. He submitted proof to show that leafroll was transmitted in the field by capsid bugs (*Calocoris bipunctatus*) and jassids (*Typhlocyba Ulmi*) as well as by aphids (*Myzus Persicae*).

Wortley (58) studying this disease, using chiefly the Bliss variety of potato, calls attention to the characteristic symptoms as they occur in this variety in Bermuda, namely, the rolling and rigidity of the leaves, particularly of the lower leaves; the development of tubers close to the stem; the dwarfing of vines; the slight yellowing of foliage; and decreased tuber production. These symptoms, together with the upright habit of the vine and the tendency of the seedpiece to remain without decay in the ground, constitute the chief characteristics of

the disease. Wortley questions the dependability of the "primary" symptoms as described by Quanjer and also gives experimental field evidence to refute the latter's claim that leafroll may be transmitted by means of the soil.

Quanjer (42) claimed to find a characteristic and dependable symptom of leafroll in a necrosis of the phloem which he further designated as a lignification of the tissues of stems and foliage. His conclusions have met with opposition from certain sources, yet, nevertheless, they have received a large measure of support and acceptance among plant pathologists. Von Brehmer (55) supports Quanjer's view that phloem-necrosis is a symptom associated with leafroll, while Esmarch (15) and Schander and Tiesenhausen (48) state that phloem-necrosis is not a dependable symptom since it occurs in cases of disease other than leafroll and also in healthy plants.

Artschwager (2), in 1918, failed to find distinct correlations between internal and external symptoms, having studied both European and American material. He also failed to find a lignification of the phloem such as had been claimed by Quanjer. However, in a later study (4, p. 243) he reverses this decision and states that "stem sections of a typical leafroll plant exhibit, as diagnostic internal symptoms, a necrosis and lignification of the phloem groups."

The specific purposes of the present study are:

1. To trace the development of phloem-necrosis in the tuber, showing histological, cytological and microchemical changes in comparison with normal structure.

2. To report the experimental production of tuber phloem-necrosis under controlled conditions in the field.

PRELIMINARY FIELD STUDIES

Following previous studies upon potato degeneration diseases which extended over several years (17), the writer undertook in 1926 to study potato tuber necrosis. Abundant material was available, due to an unusual development of the disease in the stock of a large Vermont grower, and plantings were made in an experimental plot in Burlington. The results of this planting, in part previously reported (18), furnished additional confirmation of the hypothesis, previously advanced by Schultz and Folsom (49), that there is definite correlation between net-necrosis and leafroll.

The 1926 trial gave further confirmation of the evidence that

necrotic tubers consistently produce leafroll plants. In the first three rows at the right of the plot (plate I, fig. 1), plantings of necrotic tubers in tuber units resulted in a poor stand, many weak plants and 100 percent of leafroll hills. The necrotic seed tubers for these rows were selected from sacks of commercial stock showing about 15 percent of necrosis. Several rows were also planted with tubers just as they came from the sacks and without reference to the presence of necrosis. The result in the latter case was 25 percent of leafroll. These rows are shown at the left of the necrotic rows (plate I, fig. 1). In connection with other evidence as to the origin of the trouble, it is to be noted that no necrosis developed in tubers from leafroll hills of the first three rows, while in the progeny of the adjacent three rows, containing both healthy and leafroll plants, considerable necrosis appeared. Tuber units of leafroll plants from necrotic tubers are shown in plate I, fig. 2, and in plate II, fig. 2, while in plate II, fig. 1, is pictured a unit of healthy hills from the rows containing 25 percent of leafroll.

Fixed material from the tubers used in this experiment and from vines, stolons and young tubers of plants grown in the plot, formed the basis for the histological studies. Subsequently material has been drawn from other lots, though largely from Vermont sources as a matter of convenience. The studies were continued during the winter of 1926-27 and during the following summer at the University of Wisconsin, where significant field results were secured and where much of the histological work was done.

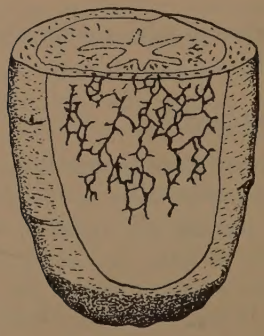

FIG. 1. Diagrammatic view of portion of necrotic tuber. The removal of epidermis and outer cortex reveals the brown, anastomosing strands of the necrotic phloem.

DESCRIPTION OF NET-NECROSIS

The significance of the term net-necrosis is most clear when the skin and outer cortex of an affected tuber are removed, exposing the browned phloem strands of the cortex—the outer phloem of the tuber as shown. These strands, composed of small groups of sieve-tubes and companion-cells, form a branching and anastomosing system which is continuous both in cortex and medulla from stem to apex.

With the development of the disease the cells of these strands die and turn brown. The browning which begins at the stem end gen-

FIG. 2. Three groups of tuber phloem cells showing the earliest observable indications of necrosis. (A) External phloem, immature, with several dead nuclei and some adjacent parenchyma cells. (B) Cross-section of a similar strand. (C) Older strand with degenerating protoplasts in sieve-tube and companion-cells.

erally extends only part way through the tuber. In some cases the discolorations are discontinuous, occurring in more or less isolated areas; again, in seriously affected tubers, the necrosis may extend

quite to the apex, although this is not common. As the necrotic effects become more marked, phloem parenchyma cells adjacent to the sieve-tubes also become involved, their walls showing microchemical changes to a distance of from one to several cells from the sieve-tube strands

FIG. 3. Necrotic area in internal phloem of necrotic tuber, showing several sieve-tube strands and adjacent phloem parenchyma with necrotic walls as indicated by the darker shading. The cells on the outer margin are normal.

(text fig. 3; plate X). Early stages of necrosis often show areas of various sizes which present a water-soaked appearance with necrotic strands at their centers. This condition, which may be due to the transformation of storage starch into sugars, is accompanied by changes in cell walls and contents which finally result in the hardening of affected walls by suberization and lignification, the dying and turning brown of protoplasmic contents and often the collapse of groups of cells at the centers of the necrotic areas. These spots therefore, as seen in cross-section, always include one or more of the sieve-tube strands and in addition a surrounding region, generally not more than two or three cells thick, of affected phloem-parenchyma cells. Large

necrotic areas may result from the fusion of several of the small spots. An interesting feature that has been repeatedly seen in this connection, but only observable under the microscope, is that the diseased areas are often cut off from surrounding healthy tissue by one or more layers of cork-cambium cells with suberized walls (text fig. 7).

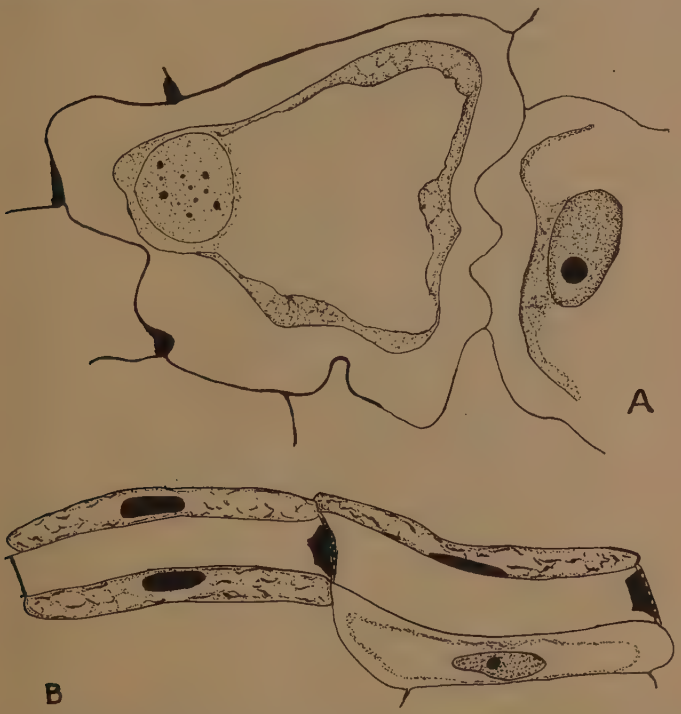

FIG. 4. (A) Parenchyma cells of tuber at the margin of a necrotic center are changed as indicated by deeper staining and breaking down of the nucleolus. (B) Sieve-tube and companion-cells from apparently healthy tuber. Nuclei in companion-cells are dead. The condition is similar to very early stages in net-necrosis.

Net-necrosis can not ordinarily be recognized without cutting the tuber. However, in the case of severely affected tubers the discoloration may be seen through the skin at the stem end or to such a distance from the stem as the necrosis is serious. Necrotic tubers are characterized, as are leafroll tubers, by the tendency to remain firm during the storage period under conditions which result in the wilting of normal tubers and seed pieces are inclined to remain hard and undecayed in the soil.

HISTOLOGICAL STUDIES

The histological studies involved in the present investigation have been pursued with two objects in view; one, such a consideration of normal tuber tissues, particularly the phloem, as would effectively contribute to an understanding of the pathological changes occurring in net-necrosis, and the other an investigation of the cell and tissue changes associated with the development of this disease.

FIG. 5. Two necrotic areas in outer phloem opposite xylem groups and separated from them by lines of cambium. Both xylem and necrotic phloem are stained deeply by the triple stain with the addition of iodine. The necrotic area at the right shows the tearing out of necrotic walls characteristic of older diseased spots.

Green Mountain potatoes drawn chiefly from Vermont sources were used. Tissues were fixed in Flemming's fluid (medium and weak) and cut about 10 to 15 μ thick. Several stains were tried, the two giving most satisfactory results being Haidenhain's iron-alum-hæmatoxylin and a modified triple stain in which Gram's iodin was introduced between an anilin solution of gentian violet and a solution of orange G in clove oil.

Since these studies have confirmed the belief previously expressed by Schultz and Folsom (49) that net-necrosis is a tuber phloem-necrosis, it is pertinent to consider the facts with reference to the structure and function of normal phloem before discussing the situation in the necrotic tuber.

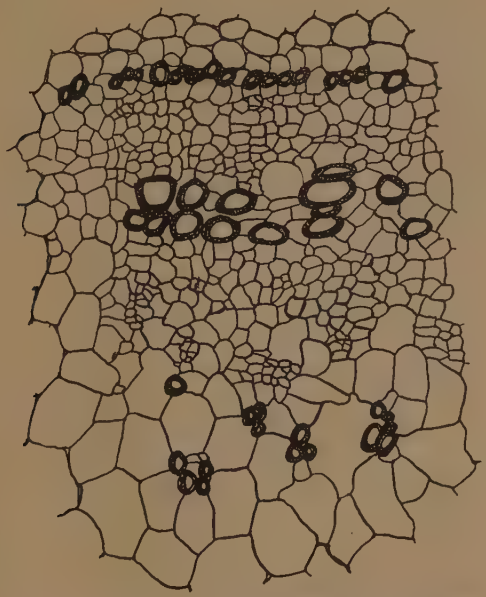

FIG. 6. Portion of stolon cross-section showing xylem cells, external and internal phloem, outer and inner pericycle and portions of cortex and pith. A large portion of the potato tuber is apparently developed by the activity of primary tissues in the regions of the inner phloem.

NORMAL TUBER STRUCTURE

Since the potato tuber is a stem structure and formed by the enlargement of the stolon tip, we may trace the development of the several regions of the mature tuber from the corresponding regions of the stolon. The epidermal and subepidermal layers give rise to the periderm which consists of cork and cork-cambium layers. The cortex of the stolon becomes that of the tuber. However, its area compared with the other tissues of the tuber is relatively much less than in the stolon. It has been shown by Esmarch (15) that much of the increase in tuber volume is due to increase in cell size, which, as noted by

Artschwager (6), may amount to 64 times that of the tissues of a small tuber. The volume increment applies particularly to the cortex and pith.

In the mature tuber the phloem strands which are concerned in net-necrosis are distributed in two regions, the outer in the exterior regions of the stele within the cortex, and the inner distributed throughout the region within the vascular ring proper and outside the pith. This distribution of outer and inner phloems and the relation of inner phloem and pith regions are illustrated in plate VI, fig. 1. The regions of the stolon which by meristematic activity have given rise to the phloem-bearing tissues are shown in plate IX, fig. 1, and in text fig. 6. So far as the outer phloem is concerned, its distribution is accounted for largely by the meristematic activity of pericycle and phloem-parenchyma cells. The activity of these tissues results in extending the stele out into the cortex so that these two regions become nearly or quite indistinguishable. In the mature tuber primary phloem strands may be found very close to the periderm. There is little cambial activity in the tuber but a small amount of secondary phloem may be formed. The inner phloem strands which in the stolon are within the xylem of the vascular ring, but close to it (plate IX, fig. 1), become diverted as the stolon tip expands to form the tuber through the activity of adjacent procambial cells. In the ontogeny of the tuber it is evident that these latter cells are responsible for the development of new sieve-tube strands, adjacent phloem parenchyma and storage parenchyma, which together make up the islands of darker tissue surrounded by pith rays, as is illustrated in plate VI, fig. 1.

Reed (47) claims that the pith and the perimedullary zone contribute equally to the formation of new tuber tissue. Artschwager (6) opposes this claim, calling attention to the fact that in a mature tuber all the tissues of the central cylinder except the most central portion (the pith) are traversed by phloem strands. He also disposes of DeVries' claim that the tuber is formed largely by the activity of the cambium, pointing out that the amount of secondary elements added as a result of cambial activity is insignificant.

The writer's studies on the point of tuber development largely confirm Artschwager's conclusions that the procambial cells of the perimedullary zone furnish much of the expansion in the swelling stolon tip. However, a critical examination of a thin slice of normal tuber (plate VI, fig. 1) shows the central pith core with its numerous branches forming a sort of network in which the phloem areas of

various sizes appear as islands, each possessing one or more sieve-tube strands. In plate VI, fig. 1, the sieve-tube strands may be recognized as faint light dots. Such an examination suggests that the pith comprises a considerable proportion of the tuber, at least 25 percent and perhaps more.

It seems that in the early stages of stolon tip enlargement there is much meristematic activity in the pith region but that in the later growth of the tuber the activity is more largely in the perimedullary zone. The early rapid expansion of pith cells causes a swelling of the stolon tip and a consequent outward divergence of the vascular tissues, including xylem and outer and inner phloem. Later activity in the procambium cells surrounding the inner phloem strands results in the expansion of tissues in this region and the consequent divergence of the strands away from the vicinity of the cortex and toward the pith.

PHLOEM DEVELOPMENT AND CARBOHYDRATE TRANSPORTATION

The potato stolon possesses a system of bicollateral bundles, this type of bundle being one in which there are two regions of phloem, one outside and the other inside the xylem. The potato tuber is a stem structure which is developed by the expansion of tissues at the stolon tip and exhibits regions of tissue largely homologous with those of the stolon. The epidermis of the stolon has been replaced by the periderm of the tuber and within the narrow cortex the great portion of the tuber is made up of internal phloem, storage parenchyma and pith.

The tuber phloem, although almost exclusively primary, is not subject to the crushing and obliteration common in many dicotyledonous stems as the result of the production of secondary phloem. In general the sieve-tube strands, which are often devoid of accompanying companion-cells, remain functional until tuber growth is complete. In the mature tuber, however, normal degeneration of phloem is found during the storage period. This type of degeneration is shown in text fig. 4, b, and should be borne in mind in connection with observations of the early stages of net-necrosis.

The origin and meaning of the bicollateral system of vascular bundles in plants in general seems to be in question. According to Wordsdell (57) medullary phloem is considered to represent a vestigial structure, being the remnant of a former system of medullary vascular bundles in which the xylem has disappeared. Scott and Brebner (52) in

discussing bicollaterality in the angiosperms consider this feature as of great systematic value and note particularly its physiological importance. They suggest the advantage of the sheltering within the woody cylinder of a portion—often the larger portion—of the delicate phloem and also the fuller utilization of the pith area. The latter point is clearly applicable to the situation in the potato tuber. Haberlandt (20) suggests that "the duplication of the phloem (in bicollateral bundles) should probably be attributed to an increase in the physiological demands made upon this section of the conducting system, a view that is supported by the fact that stems provided with bicollateral bundles frequently contain isolated accessory phloem strands as well."

Whatever may be the history of the origin and development of a bicollateral vascular system, such as occurs in the Solanaceæ and other groups, it seems fairly obvious that the divergent and interlacing phloem strands of the potato tuber show a significant correlation between position and distribution and the function which they perform. Here the function of the sieve-tube strands and perhaps also of the adjacent phloem parenchyma is chiefly the transportation of soluble carbohydrates to the storage tissues. The xylem is a negligible factor and all necessary conduction is performed by the phloem. Haberlandt (20, p. 332) cites the view put forward by Nägeli that the function of the sieve-tubes is conduction of plastic materials such as protein compounds and states that more recently Czapek has endeavored to prove that the sieve-tubes are also instrumental in transporting carbohydrates. Haberlandt believes, however, that the transfer of carbohydrates takes place mainly in the conducting parenchyma.

Eames and MacDaniels (13, p. 200) state phloem function as follows: "Taken as a whole, phloem has to do with the conduction of elaborated food products, both protein and carbohydrate, and possibly with mineral nutrients as well. Just which substances, however, move in the sieve-tubes and which in the parenchyma is not known. From the fact that the proportions of conducting parenchyma tissue to sieve-tubes show such great variation, and that the latter are almost or quite lacking in some cases, as in slender vascular bundles and in primary phloem of woody plants, it may be inferred that parenchyma under some conditions may perhaps perform the functions of sieve-tubes, or that the functions of the two types of cells may be interchangeable."

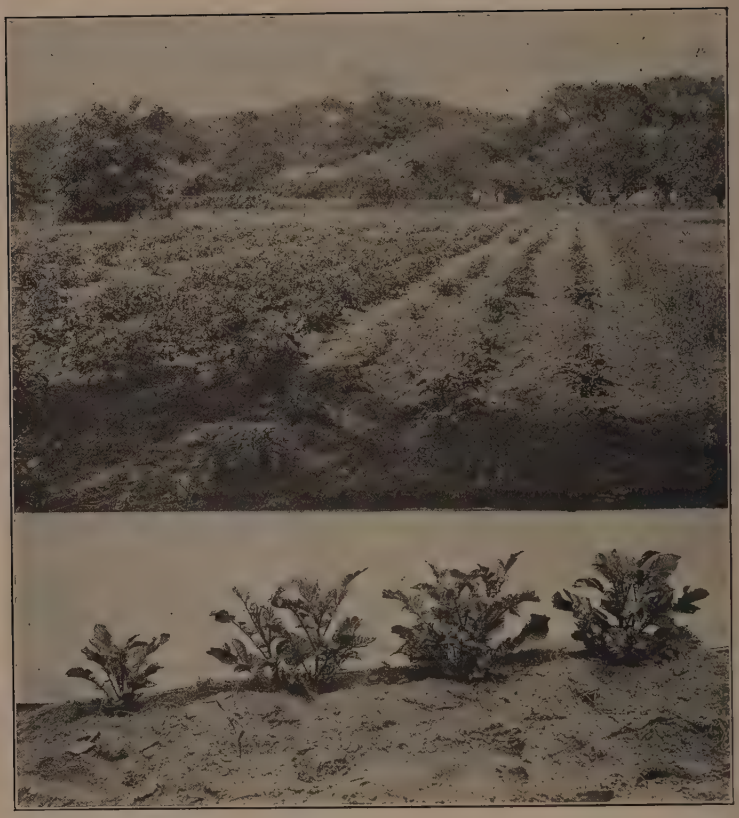

PLATE I, FIG. 1 (*upper*).—Experimental field at Burlington, Vt. The three rows on the right, planted in tuber units from necrotic tubers, contained 100 percent leafroll plants. At the left of these are three rows with only 25 percent of leafroll, the seed tubers in this case showing 10-15 percent of necrosis. FIG. 2 (*lower*).—A tuber unit of leafroll plants from necrotic tuber. From the field shown above.

PLATE II, FIG. 1 (*upper*).—Tuber unit of healthy plants in experimental field at Burlington; and from the same strain and lots as the necrotic tubers producing leafroll units, one of which is shown below. FIG. 2 (*lower*).—Tuber unit of leafroll plants from necrotic tuber. A more vigorous unit than that shown in plate I, fig. 2.

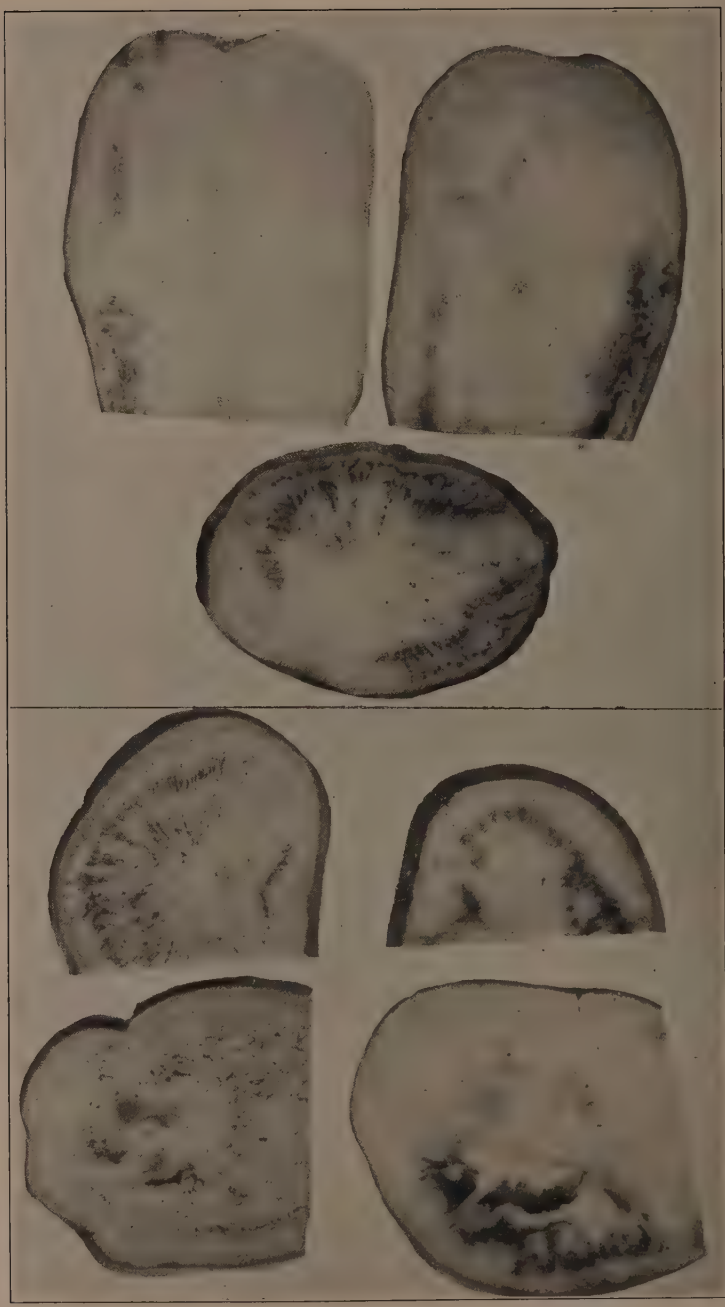

PLATE III, FIG. 1 (*upper*).—Slices from a Green Mountain tuber showing characteristic net-necrosis. FIG. 2 (*lower*).—Slices from Green Mountain tuber showing two types of frost-necrosis.

PLATE IV, FIG. 1 (*upper*).—Four plants grown from necrotic tuber, showing characteristic leafroll. FIG. 2 (*lower*).—Four healthy plants grown from tuber showing slight stem-end browning. The discoloration was not due to net-necrosis, but might easily be confused with its early stages.

PLATE V, FIG. 1 (*upper*).—Two plants (at left) from necrotic tuber, showing leafroll; and one plant (at right) from healthy tuber and showing normal foliage. FIG. 2 (*lower*).—Cages used at Madison, Wis., for experiments with leafroll infection by aphids.

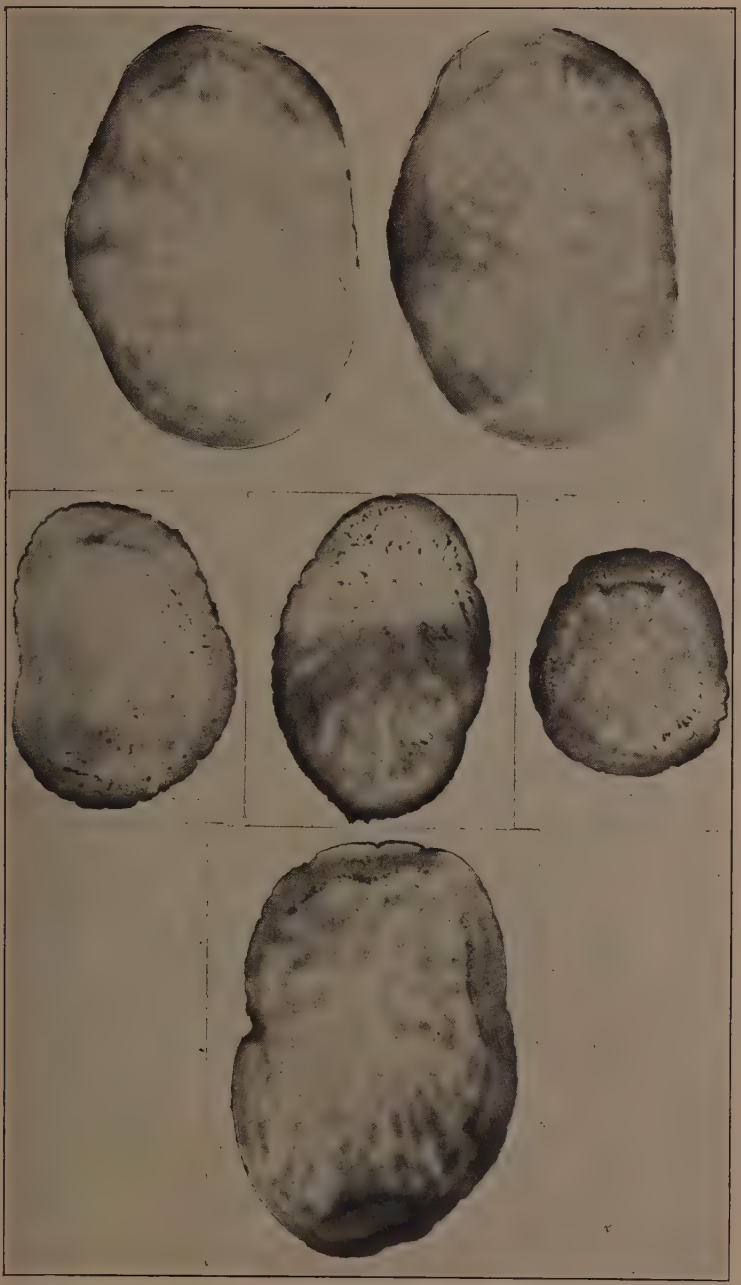

PLATE VI, FIG. 1 (*upper*).—Slices from normal Green Mountain tuber grown in check cage No. 1, Wisconsin plot. Aphids were excluded, foliage remained normal and no necrosis appeared in the tubers. FIG. 2 (*lower*).—Slices from four necrotic tubers produced in the several cages where the caged normal plants were parasitized with aphids from caged leafroll plants.

PLATE VII, FIG. 1 (*upper*).—Healthy plants grown from tubers produced in check cage No. 1, where the vines remained normal and no necrosis developed in the tubers. FIG. 2 (*lower*).—Leafroll plants grown from necrotic tubers produced in cage No. 2, where, as in the four other cages, aphids from caged leafroll plants were colonized on healthy vines.

PLATE VIII, FIG. 1 (*upper*).—Leafroll plants from tubers grown in experimental plot at Madison, Wis. Pot at left from non-necrotic tuber of leafroll hill in leafroll row. Pot at right from necrotic tuber of plant originally healthy but grown adjacent to leafroll row. FIG. 2 (*lower*).—Three plants all showing conspicuous leafroll symptoms, from necrotic tubers produced in hills originally healthy but in rows adjacent to leafroll.

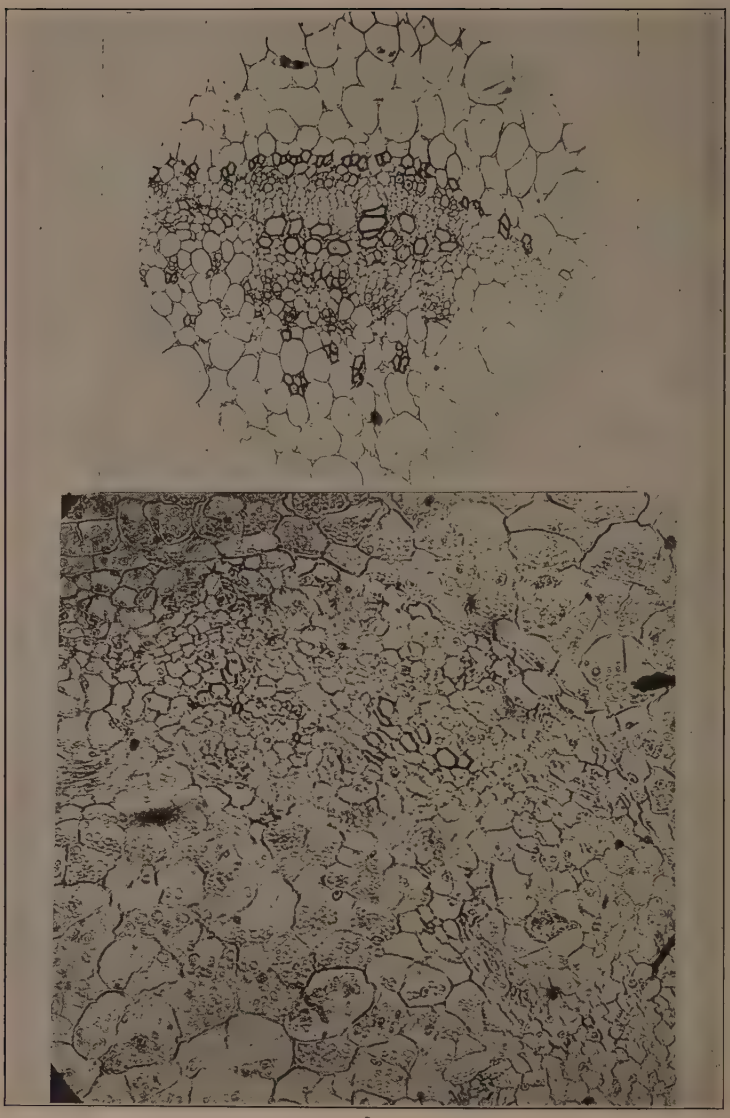

PLATE IX, FIG. 1 (*upper*).—Portion of cross-section of stolon of Green Mountain potato showing inner cortex, endodermis, pericycle with thickened walls, external and internal primary phloem groups, xylem, internal pericyclecells and pith region. FIG. 2 (*lower*).—Section from very small tuber (size of pea) showing line of endodermis and pericycle and the vascular tissue within, where rapid expansion is resulting in divergence of xylem and internal phloem groups.

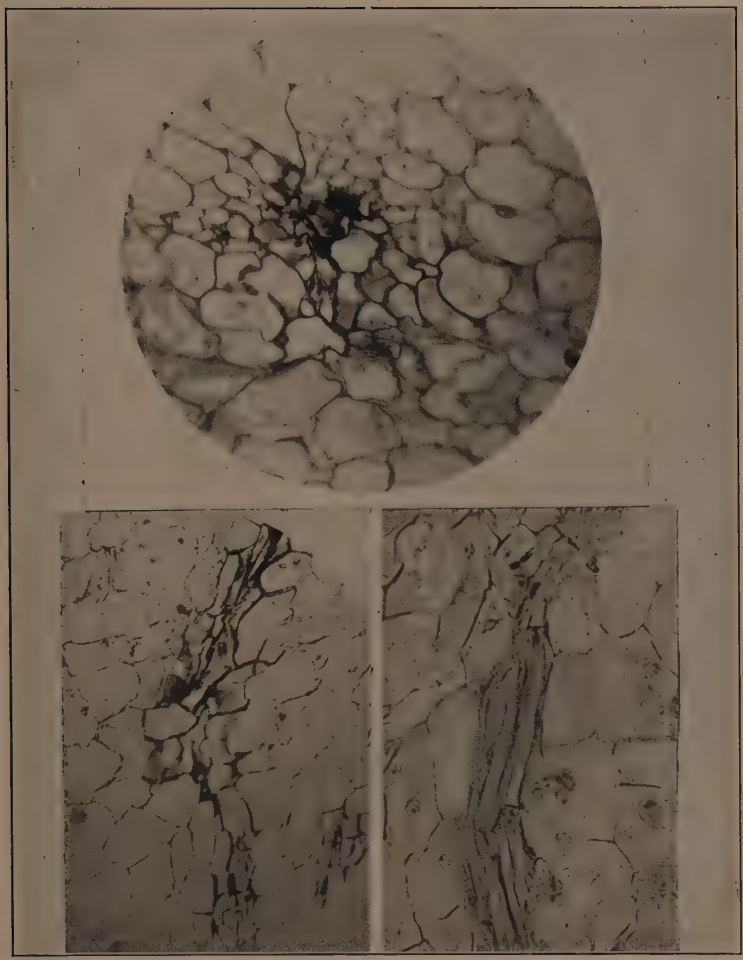

PLATE X. A (*upper*).—Necrotic spot in Green Mountain tuber, showing a central disorganized area including sieve-tube groups and a surrounding area of parenchyma cells whose walls are suberized and lignified and stained more heavily than the normal walls. B (*lower left*).—A necrotic phloem strand in longitudinal section. C (*lower right*).—A normal strand from healthy tuber.

EXPLANATION OF THE FIGURES OF PLATES XI AND XII

All figures were drawn with a *camera lucida* from razor sections of fresh material, stained as indicated in each instance. A Zeiss microscope with No. 12 compensating ocular and 4 mm. apochromatic objective was used.

PLATE XI

FIG. 1. Necrotic spot with connecting sieve-tube strand. The section was treated with strong potash solution and the shaded areas were stained a characteristic sulphur yellow. The center of the necrotic spot was a brownish yellow. (a) One of the necrotic cells showing the sharp limitation of the staining reaction. (b) Adjacent normal parenchyma cell. (c) Sieve-tube strand, also yellow.

FIG. 2. Necrotic spot showing formation of cork cambium cutting off the diseased area from surrounding tissues. This section stained with strong KOH gave the characteristic yellow reaction in the marginal cells, while the central area where cell walls are broken down is much darker, being a yellowish brown.

FIG. 3. Cross-section of necrotic strand treated with potash. Walls and intercellular substance are yellow. This is an earlier stage than that of fig. 2. Walls are thickened and enlarged intercellular spaces are filled with a gummy substance which is very resistant to all cell wall solvents.

FIG. 4. This section, showing a spot similar to that in fig. 3, except that it is nearly surrounded with a cork-cambium, was treated with alcoholic HCL for 24 hours, then stained with methylene blue 1-5 minutes. The resulting color is a clear malachite green, apparently due to a combination of the original yellowish color of the spot with the methylene blue. The presence here of calcium pectate is indicated.

FIG. 5. Necrotic spot showing disorganized center and connecting sieve-tube strand; treated with acid alcohol 24 hours and methylene blue 10-15 minutes. The necrotic spot, particularly the marginal cells and the sieve-tubes, are stained a clear blue, of a shade between cobalt and azure. In this reaction, as in other instances, there is a greenish color in the walls immediately around the necrotic center, probably due to the yellow-blue combination. This stain, also, shows the presence of large amounts of insoluble pectic substance, probably calcium pectate in the necrotic walls.

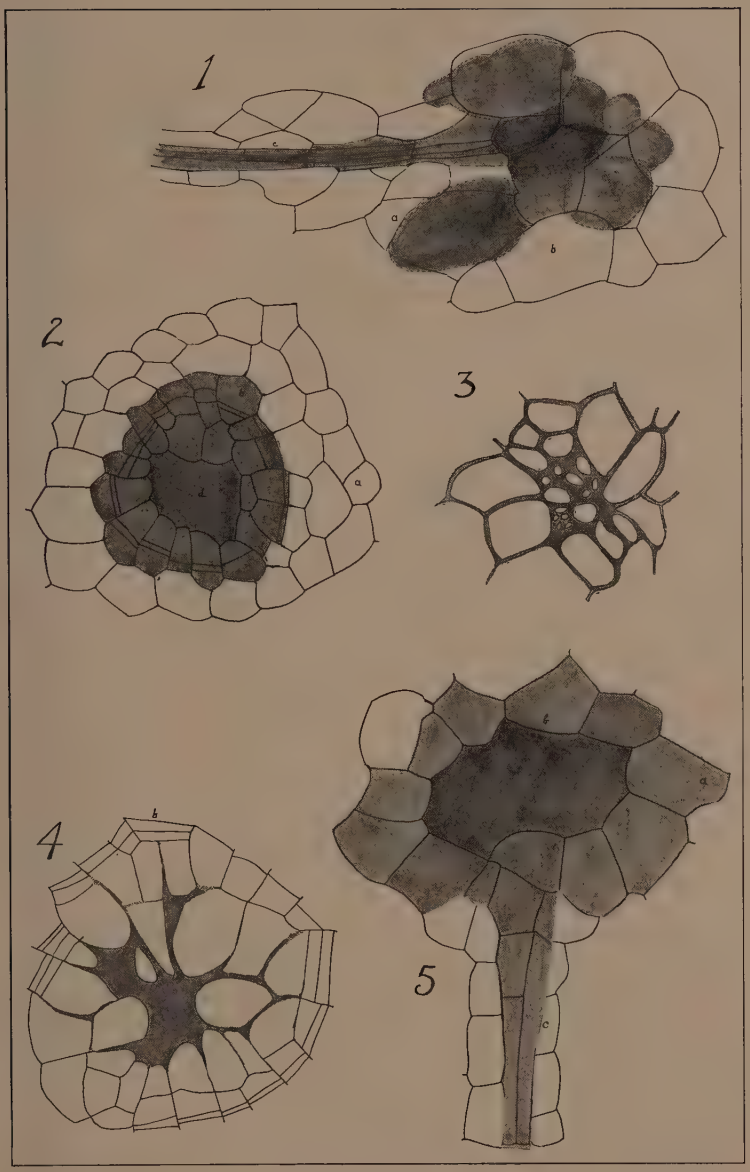

PLATE XI

Plate XII

Fig. 1. A necrotic spot with connecting sieve-tube strand adjacent to several xylem vessels with coarsely reticulate, almost spiral thickenings. Stained with phloroglucin, the center of the necrotic area is yellow, while the sieve-tubes and the xylem vessels are reddish-violet showing the presence of lignin. The walls of the parenchyma cells around the margin of the spot are also violet in color.

Fig. 2. Cross-section of a vascular bundle from a necrotic tuber, stained with phloroglucin and HCL. The xylem walls are reddish-violet, as are also the walls of the parenchyma cells adjacent to the yellow necrotic center. This gives a further indication of lignin in the necrotic walls.

Fig. 3. A necrotic spot whose central area is largely disorganized, stained with Sudan III for suberin. The color reaction is a reddish-brown. The stain is clear cut as to the margins of the stained areas, the darker center being a cinnamon-brown, while the marginal cells are rufous-red. Characteristic rod-shaped crystals in more or less stellate groups are abundant and are presumably crystals of certain fatty acids.

Fig. 4. Section stained with concentrated sulphuric acid-methylene blue. Sections were treated for several minutes with strong sulphuric acid, then washed and stained with methylene blue. Cellulose walls are dissolved but necrotic walls persist. Necrotic spots are stained a pale blue in the marginal cells, shading to a darker antwerp blue in the center. Adjacent necrotic sieve-tubes are also stained light blue and a similar reaction is seen in the cork layers of the skin. This reaction indicates again, as in plate I, figs. 4 and 5, the presence of pectic substances.

Fig. 5. Section of necrotic spot treated with cuprammonia to dissolve cellulose, then stained with ruthenium red for the presence of pectin. A delicate red stain in the walls throughout the section results. The reaction is strongest in the necrotic walls, while the center of the necrotic area is stained a reddish-brown, which is essentially the reaction with cupprammonia alone. An accumulation of pectic substances is revealed in this test and we have also another instance of the cork-cambium surrounding the necrotic area.

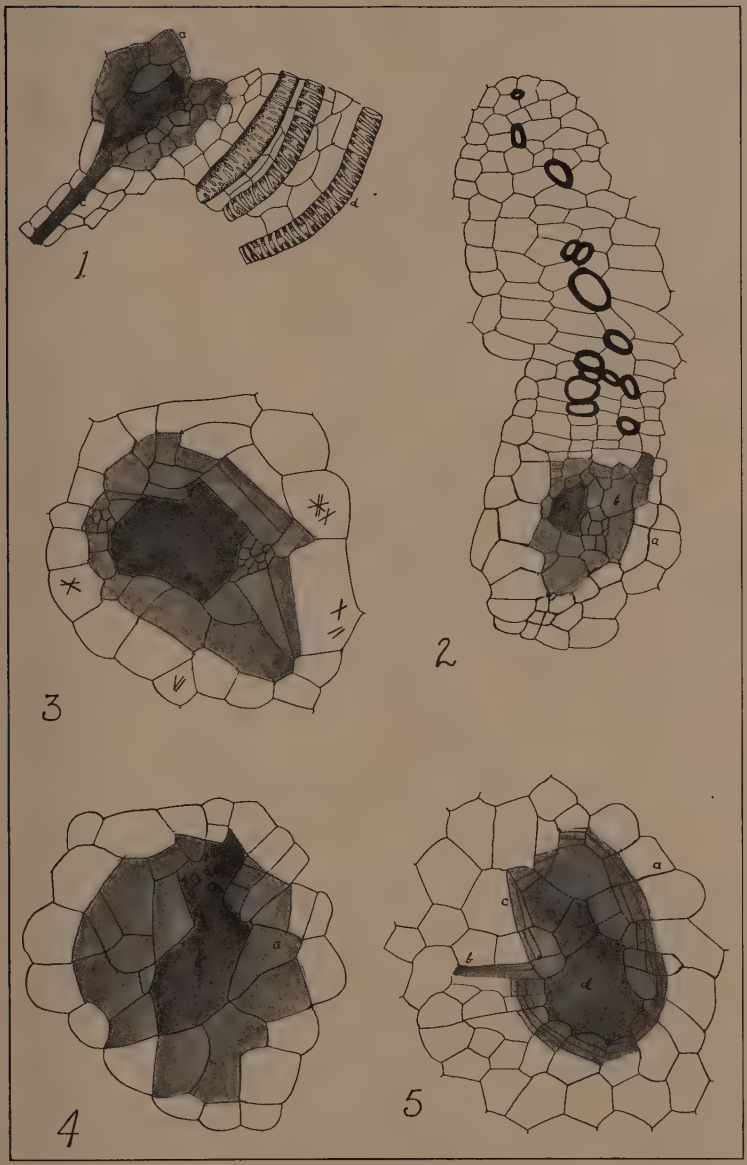

PLATE XII

STRUCTURE OF THE NECROTIC TUBER

Conspicuous evidence of phloem-necrosis in the tuber is first found at the stem or basal end, where in its initial stages it may easily be confused with browning of the vascular tissues due to fungi. However in its more advanced stages, tuber phloem-necrosis is quite characteristic in that it is confined to the phloem strands (sieve-tubes, companion-cells and adjacent phloem-parenchyma) and develops progressively from the stem toward the apex. The first phloem strands to be affected by net-necrosis are those of the outer phloem and the strands of inner phloem adjacent to the vascular ring. This situation often presents the appearance of two more or less parallel lines of necrotic discolorations extending part of the way from the stem to the apex (plate VI, fig. 2). Later in the storage period, or in cases of more serious infection, the necrotic effects may be seen in strands of phloem more distant from the vascular ring. This progressive spread in necrotic effects seems logical, since the phloem strands toward the center of the tuber, like those at the apex, are of later origin than those in the vicinity of the vascular ring. The fact that the necrotic discolorations are initiated at the basal and gradually are extended toward the bud end, is illustrated in plate III, fig. 1. The cross-section, showing extensive necrosis throughout, is taken from near the stem end where the phloem strands are converging. Both external and internal phloem strands are necrotic. In the longitudinal sections shown in plate III, fig. 2, the necrosis, though limited in extent, is seen in both regions.

In studying stained slides of a necrotic tuber it becomes evident that there are early indications of a toxic substance in cells of the phloem before there are discolorations visible to the naked eye. These initial evidences consist in the occurrence of dead and discolored protoplasmic contents of sieve-tubes and companion-cells (text fig. 2). Following or accompanying these earliest indications occur changes in the tissue walls. Such changes are revealed in thickened walls which are more deeply stained with hæmatoxylin or the modified triple stain, and involve one or more sieve-tube strands together with adjacent phloem-parenchyma cells (text fig. 3). Intercellular spaces are enlarged and filled with a deeply staining substance which may result from a partial solution of the walls. It appears that toxic substances initiating in the sieve-tubes have diffused outward and resulted in microchemical changes in the walls of adjacent phloem-parenchyma cells. These changes are indicated in text fig. 3 by the darker shading of the walls. The extent of outward diffusion of toxic sub-

stances is usually limited to a region of not more than two to four cells in thickness and at the margins of these areas the wall changes end rather abruptly (text figs. 3 and 4 A).

A normal phloem strand is shown in plate X, c, and a necrotic strand in plate X, b, the latter showing necrotic phloem-parenchyma walls rather than sieve-tubes.

In connection with a consideration of the initial effects of necrosis as shown in degenerating nuclei and protoplasm of sieve-tubes and companion-cells, it should be borne in mind that somewhat similar effects are also found in normal tuber phloem. Text fig. 4, b illustrates the situation in a healthy tuber in which the sieve-tubes are devoid of living contents and the companion-cells show dead nuclei. Sieve-tubes in normal tissues become dead cells under certain conditions of age and decrease in function. Unlike net-necrosis, however, this condition is not progressive to adjacent tissues and is not associated with the cell wall changes that are characteristic of that disease.

As the necrotic effects become more serious, the walls of sieve-tube strands and adjacent cells collapse and together with the cell contents are merged into a disorganized mass of gummy mucilaginous material which is deeply staining and very resistant to solvents. Such a situation as is pictured in plate X, a, is characteristic of this stage of the disease.

Considerable difficulty was experienced in making satisfactory microtome sections through the larger affected spots, on account of the rupturing and tearing of necrotic walls. These walls were quite brittle and were generally broken in cutting (text figs. 5 and 7). In the light of the microchemical studies and the conclusions derived therefrom, i. e., that these walls are impregnated with suberin, lignin and pectic substances, the brittle condition and difficult cutting are readily understood. Text fig. 7 illustrates not only the tearing out of fragile walls but also the formation of new cork-cambium walls around necrotic spots. Under certain conditions the formation of such walls cutting off the diseased areas is the usual result. These conditions have been studied and are believed to consist in (a) suberization of parenchyma walls on the margins of necrotic spots, due probably to an accumulation of oxygen, and (b) the stimulation of meristematic activity following the accumulation of soluble nutrients at the blocked walls. This explanation of the corking and of cork-cambium formation is adapted from Priestley (40) and will be referred to more fully on pages 25 and 26.

The histological studies confirmed and supplemented by microchemical tests have shown the location and nature of the initial effects of net-necrosis in the potato tuber, the characteristic reactions of such tissues to the stains used and the apparent microchemical changes in the walls together with the progressive development of necrosis from the stem toward the apex. A knowledge of the origin and distribution of normal phloem in a sound tuber makes clearer the occurrence and distribution of the necrotic strands.

MICROCHEMICAL TESTS

Various tests were employed to determine the nature and extent of the microchemical changes occurring in the tissues of the necrotic tuber. The results threw much light upon the physiological activities

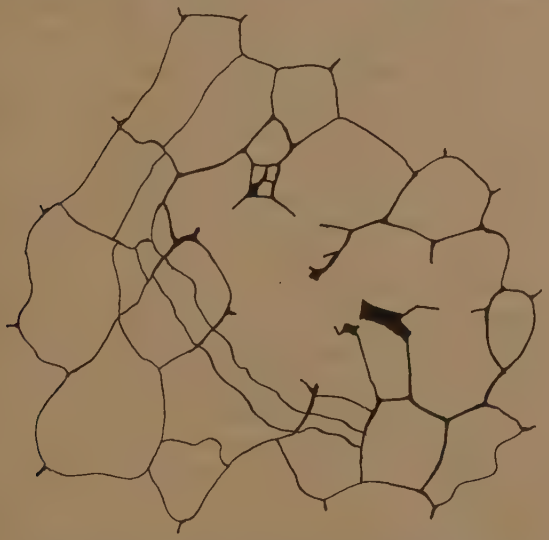

FIG. 7. Necrotic spot in external phloem showing formation of cork-cambium and the tearing out of the necrotic center where walls have become hard and brittle.

associated with the development of net-necrosis. The staining substances chiefly employed were potash solutions and Sudan III for suberin; the phloroglucin test for lignin; and methylene blue and ruthenium red for pectic substances.

In the making of these tests, thin razor sections of the tubers

were cut, treated either upon the slide or in shallow dishes and immediately studied under the microscope.

SUBERIN. *Potash Solution.* Sections thus treated show a characteristic sulphur-yellow color in necrotic walls (plate XI, figs. 1-3). Repeated tests have consistently given this tinge in the parenchyma cells, shading to an orange-brown in the darker necrotic center. Cork layers of the skin are stained a corresponding yellow, while normal parenchyma cells, phloem groups and xylem remain unstained. This reaction is interpreted as indicating the presence of suberin in the necrotic walls. The yellow color is not diffused promiscuously in the parenchyma cells adjacent to the necrotic center but is sharply limited to certain cells. This may and doubtless does mean that only these latter cells have come into contact with the toxic substance, which evidently has entered the tuber through the channel of the sieve-tubes, and, following these tissues, has diffused outward, affecting limited areas adjacent to the sieve-tubes. Often only a single parenchyma cell, or a group of two or three cells, adjacent to the sieve-tube are involved, their walls becoming so quickly and so fully impregnated with impervious material that further diffusion is effectively checked (plate XI, figs. 1 and 2).

It is evident that the toxic substances associated with this tuber necrosis move along the sieve-tubes, and that their effect upon the tissues is restricted, in general, to the sieve-tubes and a few adjacent parenchyma cells. In many instances the staining reactions indicative of a toxic substance are sharply limited to the sieve-tubes (plate XI, fig. 1, c; plate XII, fig. 1, c), while at other points, such as the ends of sieve-tube strands or the points of branching or divergence of these strands, groups of phloem parenchyma cells adjacent to the sieve-tubes are also involved. Such points furnish the characteristic cell groups figuring in a histological study of the necrotic tissues (plate XI, fig. 4, text fig. 3).

A strong aqueous solution of caustic potash, used cold, quickly gave the characteristic reaction. A three percent solution acts more slowly. Heating intensifies the color and boiling diffuses it out from the necrotic walls. The walls soften and disintegrate after prolonged treatment with dilute solutions. Since suberin and cutin are theoretically soluble under such treatment, apparently the presence of one or both these substances is indicated in the necrotic walls.

In plate XI, fig. 2, a necrotic spot stained with potash solution shows the formation of a cork-cambium cutting it off from surround-

ing tissue. Frequently, as in plate XI, fig. 1, the stain is rather definitely limited to the cells within the newly formed walls, or as previously noted, the staining substance may diffuse after the hot potash treatment. The cutting off of necrotic tissue by cork-cambium occurs often but not always. It is not generally present in the early development of the necrotic spot but is more common in advanced stages, and wherever disintegrating walls have made cavities at the necrotic centers. Probably air or oxygen accumulates which, in part at least, is responsible for the meristematic activity. A comparison of figs. 2 and 4 of plate XI gives some evidence of conditions under which cork-cambium is formed. In fig. 3, the necrotic area includes centrally located sieve-tube groups, with larger and surrounding phloem parenchyma cells. The walls are thickened and intercellular spaces are filled with a deeply staining substance very resistant to solutes of all kinds. This cell group represents the condition in which the necrotic changes are not so far advanced as to result in the collapse of walls or the occurrence of large air spaces. In fig. 2 of plate XI, however, there is a well-formed cork-cambium layer and it will be observed that the central tissues in this spot are disorganized so that no cell outlines are visible.

Sudan III. Sections stained with Sudan III show a reddish-brown color in the cell walls of the necrotic spots (plate XII, fig. 3). The stain is clear cut as to the areal margins, the center being somewhat darker resembling cinnamon-brown, while the walls of the marginal cells are red. Many crystals occur in the cells without the necrotic areas. These are slender and rod-shaped, single, in pairs or in star-shaped clusters and generally abound over the section surface after staining. They are believed to be composed of certain fatty acids.

LIGNIN. *Phloroglucin and Hydrochloric Acid.* These tests gave positive results with necrotic tubers. The necrotic center is yellow while the walls of the marginal parenchyma cells and the affected adjacent sieve-tube strands are reddish-violet, indicating the presence of lignin (plate XII, figs. 1 and 2). The lignified walls of the spiral and reticulate vessels take the same reddish-violet color. The hue soon fades from the necrotic walls but is much more persistent in the xylem walls. The same treatment applied to slices of normal tuber gave strong lignin reactions in xylem vessels but no reaction whatsoever either in sieve-tube strands or phloem parenchyma walls.

PECTIC SUBSTANCES. *Ruthenium Red.* Sections of necrotic tubers were treated with an aqueous solution of ruthenium red (1-10,000). Very little color appeared in the walls. A stronger solu-

tion (1-1,000), gave a bright red color in all parenchyma walls but no distinction was observed between necrotic and non-necrotic walls and the normally yellow necrotic centers remained yellow. Mangin (30) states, as one of the important properties of the stain, that it takes effect actively upon the gums and mucilages derived from pectic compounds without coloring those formed from cellulose or callose. When hydrochloric acid and alcohol (1-4 parts) were added to a section stained with ruthenium red the color was removed throughout, and removal was hastened by warming the slide. After washing and restaining, the parenchyma walls around the necrotic center were stained red, the surrounding tissues remaining unstained. This seems to indicate an accumulation of insoluble pectic substances in the necrotic walls, since, according to Mangin (28) acid-alcohol dissolves pectose and other soluble pectic compounds leaving the insoluble pectic acid. Hence it would seem that the pectic content of the necrotic walls is an insoluble pectic acid, and that this reaction indicates in part the nature of the changes occurring therein, constituting an added means of differentiating normal from necrotic walls.

Sections treated with cuprammonia to dissolve cellulose and then stained with ruthenium red, gave a delicate red stain in the walls throughout the section with a deeper stain in the necrotic walls (plate XII, fig. 5). The disorganized center of the necrotic area is stained a reddish-brown which is essentially the reaction with cuprammonia alone. An accumulation of pectic substance in the necrotic walls is thus revealed. Another instance of cork-cambium surrounding the necrotic area is illustrated in the same figure.

Methylene Blue. The reaction with a 1-1,000 aqueous solution is similar to that with ruthenium red in giving a generally distributed stain for pectic materials throughout the section but with little or no differentiation of necrotic walls. However, when slices are treated for one to two days with the hydrochloric-alcohol solution above indicated and then stained for one minute with methylene blue, no blue color appears in storage cell or phloem walls, but those of the cells bordering the necrotic centers are strongly stained (plate XI, fig. 4). A green color probably due to a combination of the original yellow of the necrotic spot and the blue of the stain appears in this instance. Here, again, the result indicates the presence of insoluble pectic compounds in the necrotic walls.

SOLUBILITY

The cell walls of necrotic spots resist solution when thin tuber sections are boiled in 3 percent potassium hydroxid, as do the cork layers on the surface of the tuber, but normal tissue walls are disintegrated. They also resist treatment with concentrated sulphuric acid; and this applies not only to the gummy necrotic centers but to the adjacent parenchyma cell walls, i. e., those which show some necrotic effects. When treated with 50 per cent chromic acid normal tissues are dissolved while necrotic spots with adjacent walls remain intact. Strong hydrochloric acid acting for several days disintegrates the parenchyma tissues but cork layers of the skin and necrotic spots persist. The results secured with these and other tests reveal a high degree of resistance on the part of the mucilaginous necrotic centers and the modified adjacent necrotic walls. The former are of unknown constitution, the latter contain lignin, suberin and insoluble pectic compounds.

SUBERIZATION AND MERISTEMATIC ACTIVITY IN CONNECTION WITH NECROTIC SPOTS

In attempting to explain the microchemical and other changes involved in the corking off of the necrotic areas the walls of phloem parenchyma cells adjacent to the necrotic sieve-tubes should be looked upon in the first place as wound surfaces. According to purely hypothetical theories advanced by Haberlandt (21), the stimulus resulting in meristematic activity is referred to hormones (Leptom-, Wund- and Nekro-hormones). These are held to bring about such types of meristematic activity as the production of adventitious embryos as the result of wounding. Nekro-hormones are said to be responsible for stimuli and new cell production in connection with degenerating cells and these stimuli are assumed to be transmitted to a distance of several cells from the point of stimulus.

Appel (1) has shown that the first step in healing in the case of a cut tuber surface is the deposition of a layer of suberin in the walls beneath the cut surface and confirms the statements of earlier investigators, according to Priestley (40), that the formation of suberin is definitely connected with access of oxygen.

The views of Priestley and his associates (40, 41, 56), based upon Appel's earlier studies on the physiology of wound-cork formation, seem to the writer to suggest a logical and adequate hypothesis for

explaining the formation of cork-cambium around the necrotic spots in the potato. According to these workers the conditions leading up to meristematic activity are as follows: upon wounded surfaces exposed to air there is a deposition of suberin or cutin which causes a blocking of such otherwise permeable surfaces and this results in sap accumulation followed by cell division. It was suggested by the same workers that the preliminary suberization would also involve the accumulation at the blocked surface of relatively acid substances and that between these and the living tissues a gradient of hydrogen ion concentration would be established which might be an essential factor in meristematic activity.

Lutman (27, p. 55) gives the following sequence of events in injured tuber tissue: "(1) A temporary healing of the wound by the deposition of some fat-like substance in the walls of the cells on the cut surface within 24 to 48 hours, if oxygen is present. (2) The regeneration of a new, true cork layer under this temporary one which is designed only to prevent the immediate entrance of bacteria or fungi into the interior of the tuber."

In necrotic spots where meristematic activity has been repeatedly observed resulting in the corking off of diseased areas (plate XI, figs. 2 and 4; plate XII, fig. 5; text fig. 7), the situations as revealed in histological and microchemical studies are interpreted as follows: There is first a killing of the protoplasmic contents of the sieve-tubes and companion-cells, presumably as a result of the distribution of the leafroll virus through these tissues. This effect is evident in stained slides of paraffin material before any appreciable microchemical change has occurred in the sieve-tube walls or in the adjacent phloem parenchyma (text fig. 2). Sieve-tube walls then become changed in such a way that they react strongly to suberin and moderately to lignin tests (plate XI, fig. 1, c; plate XII, fig. 1, c). There is apparently a diffusion outward of the toxic substances from the sieve-tubes to a distance of from one to three parenchyma cells. The characteristic brown necrotic strands that are conspicuous upon slicing an affected tuber (text fig. 1 and plate III, fig. 1), consist of one or more sieve-tube strands with adjacent phloem parenchyma cells. This outward diffusion of toxic substances, which apparently takes place early in the infection, is evidently effectively checked by the suberization and lignification of the walls, not only of the sieve-tubes but of the adjacent phloem parenchyma (plate XI, fig. 1). It remains to be explained why the toxic substances seem to have been in part retained

within the sieve-tubes, while more generally they have been diffused outward and have affected surrounding tissue in the restricted areas previously noted. From such observations as have been made it seems reasonable to conclude that the outward diffusion takes place more rapidly—and thus affects a wider area before corking occurs—at certain points than at others, such as points of divergence and branching of the sieve-tube strands and points where there are exposed to surrounding cells greater areas of walls of the virus carrying tissues.

In thus making use of Appel's hypothesis with reference to the initiation of suberization and applying it to the situations in the necrotic tuber, the question of a source or sources of oxygen must be considered. Two such sources suggest themselves and both may function. First, the air, which may be enclosed in the intercellular spaces and accumulate in cavities following disorganization. Second. the probable accumulation of oxygen due to oxidizing enzyms associated with the leafroll virus. The possession of a high oxidase content on the part of the leafroll potato plant has been noted by several investigators Sorauer (53) in 1908, according to Bunzel (8a), was the first to attribute leafroll to disturbances in the oxidase mechanism of the tubers. Doby (11a) in 1911-12 investigated the oxidase content of leafroll tubers and found it to be greater than in normal tubers. Bunzel (8a, 8b) found increased oxidase activity in curly-dwarf potatoes and in curly-top of sugar beet. Quanjer and his associates (43a) state that increased oxidase activity was shown in the case of potato leafroll.

Following up this point, the writer has made a number of tests for oxidase in both necrotic and healthy tubers. The former treated with an alcoholic solution of gum guiacum gave a strong greenish-blue reaction, the latter a very slight reaction. That this increased activity of oxidising enzyms may involve the release of oxygen and indirectly bring about the characteristic corking of necrotic walls and the formation of a cork-cambium around necrotic spots, is the writer's explanation of the facts herewith presented.

EXPERIMENTAL PRODUCTION OF NET-NECROSIS

In view of the considerable amount of data indicating close relationship between leafroll and net-necrosis, together with the writer's observations that tubers from known leafroll plants were consistently free from necrosis, it seemed logical to consider net-necrosis as a leafroll symptom but as one occurring only following initial leafroll

infection. Hence a trial was planned to furnish evidence on this point.

A plot of sufficient size was placed at the writer's disposal on the farm of the Wisconsin College of Agriculture and the trial carried out during 1927. The tubers were shipped promptly after digging to this Station where subsequent observations and germination tests were made.

The first plan of procedure, later modified, was to plant leafroll and healthy seed tubers in alternate rows and to secure leafroll virus transmission by means of aphids colonized upon the leafroll plants and allowed to migrate to adjacent healthy rows. Several cheesecloth cages, each covering two healthy check hills, were put in place just as the plants were sprouting.

The tubers used for planting the leafroll rows were from leafroll plants of the previous season's experimental plot and showed no signs of necrosis. The so-called "healthy" rows were planted with certified tubers from two sources (Wisconsin and Vermont) and the occasional mosaic or leafroll plant appearing was removed as soon as symptoms were manifest. However as the season advanced it became evident that no considerable infestation of aphids would be secured in the open because of weather conditions and of the unusual frequency of other insects. The procedure was at once modified so as to conduct all the work under the cloth cages. With some difficulty aphids (*Myzus Persicæ*) were colonized upon leafroll plants under cage and these virus-bearing aphids then transferred by removing the leaves upon which they were feeding and placing them upon the foliage of caged healthy plants. Five such transfers were made at intervals of a few days from August 1 to August 20 to each of five cages. Cages I, II and III were put in place as the plants were coming out of the ground while cages IV-VIII were placed after the plants were partly grown. This resulted in the presence of some leafhoppers on the vines in cages IV-VIII. The cages were fumigated but some of the invaders withstood the treatment and by their presence interfered with the aphid colonization. Cage II, put in position early and hence free from leafhoppers, produced 100 percent of necrosis. In cages V-VIII wherein leafhoppers interfered to some extent the percentages of necrosis were lessened (table I).

These field results support the hypothesis that net-necrosis is a tuber symptom following an initial leafroll infection. It occurred in percentages varying from 50 to 100 in the tubers produced in cages wherein virus-bearing aphids were introduced, while in the yields

from the 3 check cages no necrosis whatsoever was found. However, subsequent germination tests showed leafroll in plants grown from tubers produced in cage III (see table IV). This was a small cage containing only one plant while each of the other cages used contained two plants. The results from this cage should be disregarded since it is probable that the plant carried leafroll infection when caged. The other check cage containing two plants produced tubers showing no necrosis and these tubers when germinated later in the greenhouse tests (table IV) gave uniformly healthy plants.

TABLE I. RESULTS OF FIELD TRIAL IN TRANSMISSION OF LEAFROLL*VIRUS AND PRODUCTION OF NET-NECROSIS IN GREEN MOUNTAIN POTATOES

Cage number	No. of plants	Source of seed	Condition of caged plants	Treatment	Condition of progeny tubers
I	2	Wisc. certif.	Healthy	Check	No necrosis
II	2	Vt. certif.	Healthy	Aphids fr. LR Pl.	100% necrosis
III	1	Vt. certif.	Healthy	Check	No necrosis
IV	2	Vt. strain	Leafroll	Aphids	No necrosis
V	2	Vt. certif.	Healthy	Aphids fr. LR Pl.	50% necrosis
VI	2	Wisc. certif.	Healthy	Aphids fr. LR Pl.	50% necrosis
VII	2	Vt. certif.	Healthy	Aphids fr. LR Pl.	75% necrosis
VIII	2	Vt. certif.	Healthy	Aphids fr. LR Pl.	50% necrosis

*The leafroll plants of this trial were grown from seed tubers produced by leafroll plants selected in 1926. The tubers when cut for planting showed no necrosis.

TABLE II. RECORD OF OCCURRENCE OF NECROSIS IN ALTERNATE ROWS OF WISCONSIN FIELD PLOT

Row number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Condition of seed tubers	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H
Condition of progeny plants	LR	H*	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H	LR	H
Number of tubers examined October 25, 1927	25	14	..	..	..	16	..	10	..	10	..	12	..	..	..	..	10	..
Percentage showing necrosis	0	7	..	..	..	25	..	0	..	0	..	33	..	..	..	..	0	..
Number of tubers examined June 14, 1928	20	25	8	10	10	24	8	13	6	18	8	16	7	29	15	20	14	23
Number with spindling sprouts	13	7	8	5	10	4	8	0	6	2	8	4	7	6	15	6	14	12
Number with net-necrosis	0	7	0	5	0	4	0	0	0	2	0	4	0	6	0	6	0	6
Number with normal sprouts	7	18	0	5	0	20	0	13	0	16	0	12	0	23	0	14	0	11
Percentage with net-necrosis	0	28	0	50	0	16	0	0	0	11	0	33	0	20	0	30	0	26

*Vines not showing any observable leafroll symptoms during the season.

TABLE III. GERMINATION TEST IN GREENHOUSE OF TUBERS FROM ALTERNATE LEAFROLL AND HEALTHY ROWS IN WISCONSIN FIELD PLOT

Pot number	Source of seed Row number	Condition of parent plant	Condition of seed tuber	Condition of potted plant
13	1	Leafroll	No necrosis	Leafroll
14	1	Leafroll	No necrosis	Leafroll
15	3	Leafroll	No necrosis	Leafroll
16	3	Leafroll	No necrosis	Leafroll
1	2	Healthy*	Necrotic	Leafroll
2	2	Healthy	Necrotic	Leafroll
5	2	Healthy	Necrotic	Leafroll
3	6	Healthy	Necrotic	Leafroll
6	8	Healthy	Necrotic	Leafroll
7	8	Healthy	Necrotic	Leafroll
11	12	Healthy	Necrotic	Leafroll
8	14	Healthy	Necrotic	Leafroll
9	14	Healthy	Necrotic	Leafroll
10	14	Healthy	Necrotic	Leafroll

*Vines not showing any observable leafroll symptoms during the season.

TABLE IV. GERMINATION TEST IN GREENHOUSE OF TUBERS FROM CAGED PLANTS IN WISCONSIN FIELD PLOT

Pot number	Source of seed. Cage number	Condition of parent plant	Treatment of caged plant	Condition of tuber	Condition of potted plant
17	I	Healthy	Check	No necrosis	Healthy
18	I	Healthy	Check	No necrosis	Healthy
20	I	Healthy	Check	No necrosis	Healthy
26	I	Healthy	Check	No necrosis	Healthy
27	I	Healthy	Check	No necrosis	Healthy
23	II	Healthy*	Aphids from leafroll plant	Necrosis	Leafroll
25	II	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
24	II	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
19	III	Healthy	Check	No necrosis	Leafroll†
28	III	Healthy	Check	No necrosis	Leafroll†
21	IV	Leafroll	Aphids	No necrosis	Leafroll
22	IV	Leafroll	Aphids	No necrosis	Leafroll
29	V	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
30	V	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
34	VI	Healthy	Aphids from leafroll plant	No necrosis‡	Leafroll
35	VI	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
36	VII	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
37	VIII	Healthy	Aphids from leafroll plant	Necrosis	Leafroll
38	VIII	Healthy	Aphids from leafroll plant	No necrosis‡	Leafroll
39	VIII	Healthy	Aphids from leafroll plant	Necrosis	Leafroll

*Vines not showing any observable leafroll symptoms during the season.

†This should have been a healthy check like cage I. The irregular result may have been due to original leafroll infection in the seed piece or to incomplete exclusion of insects and infection during the season. In the latter case some necrosis would have been expected to occur in the progeny tubers.

‡Not all the tubers of a treated cage showed necrosis (see table I) but all produced leafroll plants, so far as tested.

The data shown in tables I and IV exhibit the significant results of the trial. These results seem to indicate that the occurrence of necrosis in tubers from caged plants was directly connected with the transfer of aphids from leafroll to healthy plants. In cage II (see table I) where 100 per cent necrosis occurred, the conditions were most favorable. The cage was placed early, as the plants were sprouting. The foliage was entirely normal, so far as could be determined, and no insects were present except the introduced aphids. This condition did not prevail in cages V-VIII wherein leafhoppers were present and where more difficulty was experienced in colonizing aphids.

In considering the status of net-necrosis in relation to leafroll, the theory may be advanced that the two are distinct, that the apparent relationship is only incidental, that there may be a virus for net-necrosis which is not identical with the virus for leafroll. The evidence, however, gathered both from field and histological studies is to the contrary. The tubers used for planting the leafroll rows in the experiment were from leafroll plants and were free from necrosis. There is no evidence that the ancestry of these or other leafroll plants may not have been associated in the initiation of their infection with tuber necrosis.

There are, on the other hand, some unexplained factors connected with the occurrence of tuber necrosis. One of these is that it often occurs in part of the tubers of a hill lot but not in all of them. Such a situation might be due to differences in tuber maturity, and, furthermore, the fact that necrosis has been observed to develop during the storage period may be associated with the maturing processes which go on during the rest period. Net-necrosis is a condition which arises during the rest period rather than during the growing period. In the present case it was found in abundance, though not in its most advanced stages, on October 25, two months after the tubers were harvested.

The data embodied in tables II and III are of incidental rather than of first importance but they furnish some additional evidence of necrosis production following initial leafroll infection. The leafroll rows from leafroll seed tubers were odd-numbered, those planted with healthy stock even-numbered. The latter showed no recognized leafroll symptoms during the season. Several species of insect parasites occurred in abundance, including leafhoppers, tarnished plant bug and flea beetle. Aphids, if present, were in very small numbers and repeated attempts to colonize the species *Myzus Persicæ* on plants of the

leafroll rows were unsuccessful. The fact of interest here was that considerable percentages of necrotic tubers occurred in the yields from the healthy rows, while no necrosis at all was found in the tubers from the leafroll rows (table II). Table III shows that necrotic tubers from healthy rows alternating with leafroll rows produced leafroll plants when germinated in pots in the greenhouse and that tubers from leafroll rows showed no necrosis but produced leafroll plants again when germinated.

Murphy (33) in Ireland claims to have demonstrated leafroll transmission by a species of leafhopper (*Typhlocyba Ulmi*) and by the capsid bug (*Calocoris bipunctatus*). On the basis of circumstantial evidence only, it seems highly probable that in this trial there was transmission in the open rows of the field plot by one or more of the insects noted as occurring in abundance, the most likely agents being the leafhopper and the tarnished plant bug.

CONCLUSIONS

1. Net-necrosis, a disease of Irish potato tubers, is a trouble involving primarily the phloem of the tuber. It is, therefore, a tuber phloem-necrosis and, as such, exhibits certain fundamental features in common with the phloem-necrosis described by Quanjer and associated with leafroll. The appearance of a network is evident when, upon removing the skin and outer cortex of an affected tuber, coarsely reticulate brown lines reveal the position and distribution of the diseased phloem strands which here include the sieve-tube bundles together with some adjacent phloem parenchyma cells.

2. Net-necrosis was described and named by W. A. Orton in 1914 and has been reported by investigators in England, Germany, France, Holland, Japan and Australia as well as in America. It is distinguished from other tuber discolorations, such as internal brown spot, sprain, frost-necrosis and stem-end browning, by certain anatomical characters; that is to say by the fact that the necrosis is limited to the phloem tissues and involves neither the xylem (except in a very incidental manner) nor the pith, and by the further fact that it occurs during the storage period, appearing only after harvest and progressing during the subsequent rest period.

3. Numerous observations by the writer and others have shown that net-necrosis of the tuber definitely correlates with leafroll, that necrotic tubers consistently produce leafroll plants, and, conversely, that necrosis occurs in tubers from plants grown adjacent to leafroll hills.

4. Net-necrosis is described as consisting in a browning of the phloem strands of the tuber, appearing first at the stem end and progressing toward the bud end. The brown strands consist of groups of sieve-tubes together with adjacent affected phloem parenchyma cells. These diseased strands, when observed after removing the outer cortex, appear as a brown network.

5. As to the development of the tuber from the stolon tip, the writer's observations agree largely with those of Artschwager that the procambium cells of the perimedullary zone furnish much of the expansion connected with tuber growth, another important factor being cell enlargement without division in the regions of pith and outer medulla. The pith with its branches is believed by the writer to constitute at least 25 percent of the volume of the tuber and to be a larger factor in tuber structure than has been generally considered.

The potato tuber develops through the expansion of the tissues of the stolon tip and its tissues are largely homologous with those of the stolon. The latter possesses a system of bicollateral bundles in which there are two regions of phloem, one outside and the other inside of the xylem. Very little cambial activity exists in the tuber and it contains little secondary xylem or phloem. Tuber expansion involves the extension of phloem strands in regions farther removed from the vascular ring and of later development. In its ontogeny there is evidently a definite correlation between the position and distribution of the phloem strands and the function which they perform.

6. Phloem-necrosis is first seen at the stem or basal end. It is characteristic in that it is confined to the phloem strands, involving sieve-tubes and companion cells and adjacent phloem parenchyma. In the order of development of the disease, the tissues first affected are the outer phloem and the strands of inner phloem adjacent to the vascular ring. Later on necrotic effects may be seen in phloem strands more distant from the vascular ring and consequently of subsequent development.

7. The earliest indications of necrotic changes in the tissues are the occurrence of the dead and discolored protoplasmic contents of sieve-tubes and companion cells. In due time entire phloem groups become brown and larger areas result from the merging of several such groups. Cell walls become changed chemically and disorganized and necrotic centers consist of gummy masses which are very resistant to solvents.

8. A common feature of the necrotic spots is the formation of

new cork-cambium walls surrounding the necrotic centers. This is believed to be due to the suberization of parenchyma walls resulting from an accumulation of oxygen and to the stimulation of meristematic activity following the accumulation of soluble nutrients at the blocked walls.

9. Microchemical tests show that the changes in the necrotic walls involve accumulations therein of suberin, lignin and pectic substances and strong resistance to cell wall solvents.

10. The results of field trials conducted at Madison, Wis., during 1927 seem to confirm the writer's hypothesis that net-necrosis is a first season tuber symptom following initial infection of a healthy plant with leafroll virus. Aphids (*Myzus Persicae*) were used for transmitting the virus, being colonized on leafroll plants under cage and transferred to caged healthy plants. Necrosis occurred in significant percentages in the treated cages while none whatever occurred under similar conditions in a check cage. The necrotic tubers thus secured, when germinated, produced leafroll plants while the tubers from the check cage produced healthy plants.

Considerable necrosis occurred in healthy rows adjacent to leafroll in the open field plot while at the same time tubers derived from these same leafroll rows showed no necrotic symptoms whatsoever. This fact seems to furnish further confirmation of the writer's hypothesis as to the origin of this tuber disease.

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